

WHAT IS CLAIMED IS:

1. A method of producing a glycoprotein with reduced complex carbohydrates comprising:
  - 5 a. introducing and expressing a polynucleotide encoding a glycoprotein into a mammalian cell;
  - b. culturing the mammalian cell in the presence of a lectin in an amount sufficient to obtain a lectin resistant mammalian cell;
  - c. isolating the lectin resistant mammalian cell;
  - 10 d. culturing said lectin resistant mammalian cell, expressing said glycoprotein; and
  - e. collecting the glycoprotein from said lectin resistant cells.
2. The method of Claim 1, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and  
15 wheat germ agglutinin.
3. The method of Claim 2, wherein said lectin is ricin.
4. The method of Claim 1, wherein said glycoprotein is a lysosomal hydrolase.
5. The method of Claim 4, wherein said lysosomal hydrolase is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$  -L-iduronidase,  $\alpha$  -galactosidase A,  
20 arylsulfatase , N-acetylgalactosamine-6-sulfatase or  $\beta$  -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C,  
25 Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase G<sub>M1</sub>

Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

- 5      6. The method of Claim 5, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.
7. The method of Claim 1, further comprising contacting the collected glycoprotein with a GlcNAc-phosphotransferase.
8. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises  
10      SEQ ID NO:2.
9. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2 and SEQ ID NO:7.
10. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises SEQ ID NOS:4, 5 and 7.
- 15      11. The method of Claim 7, wherein the GlcNAc-phosphotransferase is encoded by a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:1.
12. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises an  $\alpha$ -subunit and a  $\beta$  subunit, which are encoded by a nucleotide sequence  
20      comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:3; and a  $\gamma$  subunit, which is encoded by a nucleotide sequence comprising SEQ ID NO:6 or a

nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6.

13. The method of Claim 7, further comprising purifying said glycoprotein after said contacting.

5 14. The method of Claim 7, wherein after said contacting with GlcNAc-phosphotransferase the method further comprises contacting with said glycoprotein with a phosphodiester  $\alpha$ -GlcNAcase.

15. The method of Claim 14, wherein said phosphodiester  $\alpha$ -GlcNAcase comprises an amino acid sequence of SEQ ID NO:18.

10 16. The method of Claim 14, wherein said phosphodiester  $\alpha$ -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17.

15 17. The method of Claim 14, further comprising purifying said glycoprotein after said contacting.

18. A glycoprotein produced by the method of Claim 1.

19. A method of producing a glycoprotein deficient in complex carbohydrates comprising:

- 20 a. introducing and expressing a polynucleotide encoding a glycoprotein into a mammalian cell;
- b. culturing the mammalian cell in the presence of a lectin in an amount sufficient to obtain a lectin resistant mammalian cell;
- c. isolating the lectin resistant mammalian cell;
- d. culturing said lectin resistant mammalian cell; and
- 25 e. collecting the glycoprotein from said lectin resistant cells.

20. The method of Claim 19, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.
21. The method of Claim 20, wherein said lectin is ricin.
- 5 22. The method of Claim 19, wherein said glycoprotein is a lysosomal hydrolase.
23. The method of Claim 22, wherein said lysosomal hydrolase is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, 10 Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase G<sub>M1</sub> Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ - 15 fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.
24. The method of Claim 23, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.
- 20 25. The method of Claim 19, further comprising contacting the collected glycoprotein with a GlcNAc-phosphotransferase.
26. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2.

27. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises  
SEQ ID NO:2 and SEQ ID NO:7.

28. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises  
SEQ ID NOS:4, 5 and 7.

5 29. The method of Claim 25, wherein the GlcNAc-phosphotransferase is encoded  
by a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence  
that hybridizes under stringent conditions to the complement of SEQ ID NO:1.

10 30. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises  
an  $\alpha$ -subunit and a  $\beta$  subunit, which are encoded by a nucleotide sequence  
comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under  
stringent conditions to the complement of SEQ ID NO:3; and a  $\gamma$  subunit,  
which is encoded by a nucleotide sequence comprising SEQ ID NO:6 or a  
nucleotide sequence that hybridizes under stringent conditions to the  
complement of SEQ ID NO:6.

15 31. The method of Claim 25, further comprising purifying said glycoprotein after  
said contacting.

32. The method of Claim 25, wherein after said contacting with GlcNAc-  
phosphotransferase the method further comprises contacting with said  
glycoprotein with a phosphodiester  $\alpha$ -GlcNAcase.

20 33. The method of Claim 32, wherein said phosphodiester  $\alpha$ -GlcNAcase  
comprises an amino acid sequence of SEQ ID NO:18.

34. The method of Claim 32, wherein said phosphodiester  $\alpha$ -GlcNAcase is  
encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide

sequence that hybridizes under stringent conditions to the complement of SEQ  
ID NO:17.

35. The method of Claim 32, further comprising purifying said glycoprotein after  
said contacting.

5 36. A glycoprotein produced by the method of Claim 19.

37. A method of making a mammalian cell that produces glycoproteins having  
reduced complex carbohydrates comprising

a. introducing and expressing a polynucleotide encoding a glycoprotein  
into a mammalian cell;

10 b. culturing the mammalian cell in the presence of a lectin in an amount  
sufficient to obtain a lectin resistant mammalian cell;

c. isolating the lectin resistant mammalian cell;

38. The method of Claim 37, wherein said lectin is selected from the group  
consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and  
15 wheat germ agglutinin.

39. The method of Claim 38, wherein said lectin is ricin.

40. The method of Claim 38, wherein said glycoprotein is a lysosomal hydrolase.

41. The method of Claim 40, wherein said lysosomal hydrolase is selected from  
the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A,

20 arylsulfatase, N-acetylgalactosamine-6-sulfatase or  $\beta$ -galactosidase,  
iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase,  
Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -  
glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase,  
Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C,

25 Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase G<sub>M1</sub>

Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

5      42. The method of Claim 41, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.

43. A mammalian cell that produces glycoproteins having reduced complex carbohydrates obtained by the method of Claim 37.

10      44. A method of treating a patient suffering from a lysosomal storage disease comprising administering to said patient a lysosomal hydrolase in an amount sufficient to treat said disease, wherein said lysosomal hydrolase is obtained by a method comprising:

- 15      a. introducing and expressing a polynucleotide encoding said lysosomal hydrolase into a mammalian cell;
- 15      b. culturing the mammalian cell in the presence of a lectin in an amount sufficient to obtain a lectin resistant mammalian cell;
- 15      c. isolating the lectin resistant mammalian cell;
- 15      d. culturing said lectin resistant mammalian cell;
- 15      e. collecting the lysosomal hydrolase from said lectin resistant cells;
- 20      f. contacting the collected lysosomal hydrolase with a GlcNAc-phosphotransferase; and
- 20      g. contacting said lysosomal hydrolase with a phosphodiester  $\alpha$  GlcNAcCase after said contacting with a GlcNAc-phosphotransferase.

45. The method of Claim 44, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

46. The method of Claim 45, wherein said lectin is ricin.

5 47. The method of Claim 45, wherein said lysosomal hydrolase is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -  
10 glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase  $G_{M1}$  Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase,  
15 Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

48. The method of Claim 47, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.

49. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises  
20 SEQ ID NO:2.

50. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2 and SEQ ID NO:7.



51. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises  
SEQ ID NOS:4, 5 and 7.

52. The method of Claim 44, wherein the GlcNAc-phosphotransferase is encoded  
by a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence  
5 that hybridizes under stringent conditions to the complement of SEQ ID NO:1.

53. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises  
an  $\alpha$ -subunit and a  $\beta$  subunit, which are encoded by a nucleotide sequence  
comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under  
stringent conditions to the complement of SEQ ID NO:3; and a  $\gamma$  subunit,  
10 which is encoded by a nucleotide sequence comprising SEQ ID NO:6 or a  
nucleotide sequence that hybridizes under stringent conditions to the  
complement of SEQ ID NO:6.

54. The method of Claim 44, wherein said phosphodiester  $\alpha$ -GlcNAcase  
comprises an amino acid sequence of SEQ ID NO:18.

15 55. The method of Claim 44, wherein said phosphodiester  $\alpha$ -GlcNAcase is  
encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide  
sequence that hybridizes under stringent conditions to the complement of SEQ  
ID NO:17.

56. A method of producing a glycoprotein with reduced complex carbohydrates  
20 comprising:

- a. a step for introducing and expressing a polynucleotide encoding a  
glycoprotein into a mammalian cell;
- b. a step for selecting a mammalian cell expressing said glycoprotein that  
is resistant to a lectin;

- c. a step for culturing said lectin resistant mammalian cell, expressing said glycoprotein; and
- d. a step for collecting the glycoprotein from said lectin resistant cells.

57. The method of Claim 56, wherein said lectin is selected from the group

consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

58. The method of Claim 57, wherein said lectin is ricin.

59. The method of Claim 56, wherein said glycoprotein is a lysosomal hydrolase.

60. The method of Claim 59 wherein said lysosomal hydrolase is selected from

the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase  $G_{M1}$  Galglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

61. The method of Claim 60, wherein said lysosomal hydrolase is acid  $\alpha$ -glucosidase.

62. The method of Claim 56, further comprising a step for transferring a N-acetylglucosamine-1-phosphate from UDP-GlcNAc to said glycoprotein.

63. The method of Claim 62, further comprising a step for purifying said glycoprotein comprising a N-acetylglucosamine-1-phosphate.

64. The method of Claim 62, further comprising a step for removing an N-acetylglucosamine from said glycoprotein.

5 65. A glycoprotein produced by the method of Claim 56.